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Long-term persistence of poliovirus neutralizing antibodies in the era of polio elimination: An Italian retrospective cohort study

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ABSTRACT

Introduction: The extensive use of oral and inactivated poliovirus (PV) vaccines has driven progress toward the global eradication of wild PV2 and PV3 and the elimination of PV1 in most countries, including Italy. Although the persistence of circulating neutralizing antibodies among the vaccinated is unclear, it is estimated that > 99% of the population vaccinated according to the recommended protocol should be protected for at least 18 years.

Methods: This study evaluated the seroprevalence of anti-PV neutralizing antibodies and the long-term immunogenicity of the oral poliovirus vaccine (OPV) in a sample of medical students and residents of the University of Bari who attended the Hygiene Department for a biological risk assessment between April 2014 and October 2020.

Results: The prevalence of protected vaccinated individuals was > 90% for PV1, PV2, and PV3. Specifically, >99% of the study group was protected against PV1, > 98% against PV2, and almost 93% against PV3. Protective antibodies against all three viruses persisted for at least up to 18 years after administration of the last OPV dose, with PV1 and PV2 antibodies detected in > 95% of the participants > 30 years after the last OPV dose.

Conclusions: The childhood series of four doses of OPV guarantees a long duration of protection, despite the elimination of the virus and therefore the absence of a natural booster. However, until PV1 is completely eradicated, maximum vigilance on the part of public health institutions must be maintained.

1. Introduction

Poliomyelitis is a crippling and potentially fatal disease that is caused by polioviruses. The source of infection includes people who are symptomatically ill or who are carriers of the virus, which is also able to survive in the environment. Polioviruses entering the body via the oral cavity and can enter the brain and spinal cord. Irreversible paralysis occurs in 1 in 200 infections [1]. The three poliovirus serotypes (PV1, PV2, and PV3) show minimal heterotypic immunity between them; thus, immunity to one serotype does not produce significant immunity to the other serotypes [2].

Two type of polio vaccine are currently available. An inactivated poliovirus vaccine (IPV) was licensed in 1955 and was used extensively until the early 1960s. In 1963, a trivalent OPV (the "Sabin vaccine") largely replaced the inactivated polio vaccine (IPV) and it has since become the vaccine of choice in most countries, especially those with a high level of poliovirus endemicity. An enhanced-potency IPV was licensed in November 1987 and became available in 1988 [3]. The action of OPV is two-pronged: (i) producing antibodies in the blood (humoral or serum immunity) to all three types of poliovirus such that in the event of infection polio-induced paralysis is prevented by blocking the spread of the virus to the nervous system and (ii) by producing a local immune response in the lining of the intestines, the primary site of poliovirus multiplication. The latter mechanism has not been demonstrated for IPV [4]. Vaccine-associated paralytic poliomyelitis (VAPP) is a serious but sporadic and rare adverse event following OPV administration. Due to the risk of VAPP, in many countries in which the advanced control or the elimination of polio has been

Abbreviations: GIAVA, Regional Immunization Database; IPV, inactivated poliovirus vaccine; PAS, protective antibody survival; OPV, oral poliovirus vaccine; PV1, poliomyelitis virus 1; PV2, poliomyelitis virus 2; PV3, poliomyelitis virus 3.

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achieved, IPV has replaced OPV in the routine immunization schedule [5].

Almost all children (99%) who receive all four of the recommended doses of polio vaccine in the childhood series will have detectable antibodies [6]. Indeed, two doses of IPV are ≥ 90% effective against polio and three doses are 99–100% effective. The last dose in either series should be given after age 4 years and at least 6 months after the previous dose [7]. Serologic studies have shown that seroconversion following three doses of either IPV or OPV is nearly 100% for all three vaccine viruses, but the rate after three doses of a combination of IPV and OPV is lower [3].

The introduction of these vaccines has driven progress toward the global eradication of wild polioviruses, a millennium goal of the World Health Organization (WHO) [8]. Eradication of wild PV2 was declared on September 20, 2015, with the last reported case in October 1999, and the eradication of wild PV3 on October 17, 2019, following the last reported case in November 2012. On August 25, 2020, the WHO declared Africa polio-free for wild polio virus [9]. Cases due to wild poliovirus have decreased by > 99% since 1988, and since 2017 wild PV1 infections have been reported only in Afghanistan (14 cases in 2017, 21 in 2018, 29 in 2019, and 47 cases to date in 2020) and Pakistan (eight cases in 2017, 12 in 2018, 147 in 2019, and 73 cases to date in 2020) [10,11].

In 1964, the Italian Ministry of Health developed a campaign of mass vaccination with the Sabin vaccine, which was offered free and actively to all children between the ages of 6 months and 14 years; March, April, and May of that year were dedicated to vaccination against PV1, PV3, and PV2, respectively. In 1966, vaccination against polio became mandatory. In 1972, MOPVs were replaced by trivalent OPV. However, between 1964 and 2000, vaccination with OPV resulted in a small number of cases of VAPP. Based on ethical concerns and the favorable epidemiological context, in 2000 a sequential schedule (IPV-IPV-OPV-OPV) was introduced. In 2003, the use of live attenuated vaccine was suspended and IPV was introduced exclusively for polio vaccination during childhood [12]. The first three doses are administered to infants 3, 5, and 11 months of age using a hexavalent formula (IPV-hepatitis B- *Haemophilus influenzae* type b-tetanus-diphtheria-acellular pertussis), the fourth dose at 5–6 years of age in a tetravalent formula (tetanus-diphtheria-acellular pertussis-IPV), and a recommended fifth dose during adolescence. In 2017, the Italian government made vaccination against polio mandatory for infants and children [13]. The three-dose coverage achieved in infant cohorts during the period 2000–2019 ranged between 85.4% and 96.8% [14].

With the vaccination campaigns carried out since 1964, in 2002 Italy (together with the entire European region) was certified polio-free by the Regional Commission for the Certification of Poliomyelitis Eradication (RCC); but, in fact, no cases had been recorded since 1983 [15]. While it is unclear how long the vaccinated population will remain immune to the poliovirus, >99% of the group that received the recommended schedule should be protected for at least 18 years [7,16]. A WHO document reported strong scientific evidence for the long-term (>5–10 years) persistence of protective antibodies in ≥ 80% of the population vaccinated with ≥ 3–4 doses of OPV, and low scientific evidence for the long-term (>5–10 years) persistence of protective antibodies in ≥ 80% following ≥ 3–4 doses of IPV [17].

The aim of this study was to evaluate the seroprevalence of anti-poliovirus neutralizing antibodies in a sample of medical students and residents of the Medical School of Bari University who had been fully vaccinated with OPV. We also assess the long-term persistence of neutralizing antibodies conferred by vaccination with this form of the vaccine. The study was carried out in Apulia (southern Italy, ~4,000,000 inhabitants) >10 years since a similar study had been conducted in this region [18].

2. Methods

This was a retrospective cohort study.

According to the Italian Ministry of Health's recommendation, in April 2014 the Hygiene Department of the Bari Policlinico University Hospital implemented a biological risk prevention program for medical students and residents (physicians in postgraduate training) of the Medical School of the University of Bari. The study sample comprised students and residents who attended the Hygiene Department from April 2014 to October 2020.

Informed consent was routinely collected during clinical procedures and for each enrollee demographic data and the participant's medical history were documented. Informed consent for the use of data collected for clinical procedures for the purpose of publication was obtained from all participants. The study was conducted according to the principles of the Helsinki declaration.

The survey included only medical students and residents who during childhood had received four doses of OPV following the Italian vaccination schedule and who had not received a poliovirus booster thereafter. Those without an available vaccination history, who were never vaccinated, or who had been vaccinated with fewer or more than four doses of OPV at baseline or with IPV were excluded from the study. The vaccination status of the participants was assessed according to the Regional Immunization Database (GIAVA) [19]. GIAVA is a computerized vaccination registry that for every Apulian inhabitant records his or her vaccination history and, if needed, generates an immunization schedule.

Serum was prepared from blood samples and stored at −20 °C. Immunity to poliovirus was determined based on the ability of the serum sample to neutralize infectivity and the cytopathic effect (CPE) in cell cultures of each of the three types of poliovirus. The neutralization test was conducted in microtiter plates according to the guidelines of the WHO/Expanded Programme on Immunization (EPI) collaborative study of poliomyelitis [20]. Two-fold dilutions of inactivated sera (from 1/8 to 1/1024) were incubated in duplicate with suspensions of each of the three reference Sabin strains (PV1/Mahoney strain, PV2/MEF-1 strain, and PV3/Saukett strain) corresponding to a 100 $TCID_{50}$/0.025-ml challenge. After a 3-h incubation at 36 °C, 5% CO_2, a human heteroploid Hep-2 cell suspension (1–2 × 10^4cells/0.1 ml; MEM Earle's salts 10% FBS; 37 °C, 5% CO_2) was added to each well containing the virus-serum mixtures. A titration of each viral strain and cell controls were included. The plates were incubated at 36 °C for 5 days and then examined for the appearance of cytopathic effects (CPE) using an inverted microscope. Neutralizing antibody titer (expressed as reciprocal) was determined using the Karber formula, based on the highest dilution of serum that protected 50% of the cultures against a 100 $TCID_{50}$ viral challenge and inhibited CPE. Titers ≥ 1/8 were considered positive, as recommended by the WHO/EPI.

For every participant, patient identification data, sex, age at enrollment, dates of the routine poliovirus vaccine, and type1/2/3 anti-poliovirus antibody titer were recorded and entered into a database created with an Excel spreadsheet. The data were analysed using STATA MP16 software.

Continuous variables were described as the mean ± standard deviation and range, and categorical variables as proportions, with 95% confidence intervals (95%CIs), when appropriate. Protective antibody titers were classified as low (1/8–1/32) or high (1/64–>1/256) (in continuity with Tafuri S, 2008 [18]) and compared by sex and by age class. Skewness and kurtosis tests were used to evaluate the normality of the continuous variables, but all were normally distributed or normalizable. The Wilcoxon's rank sum test was used to compare continuous variables between sexes;

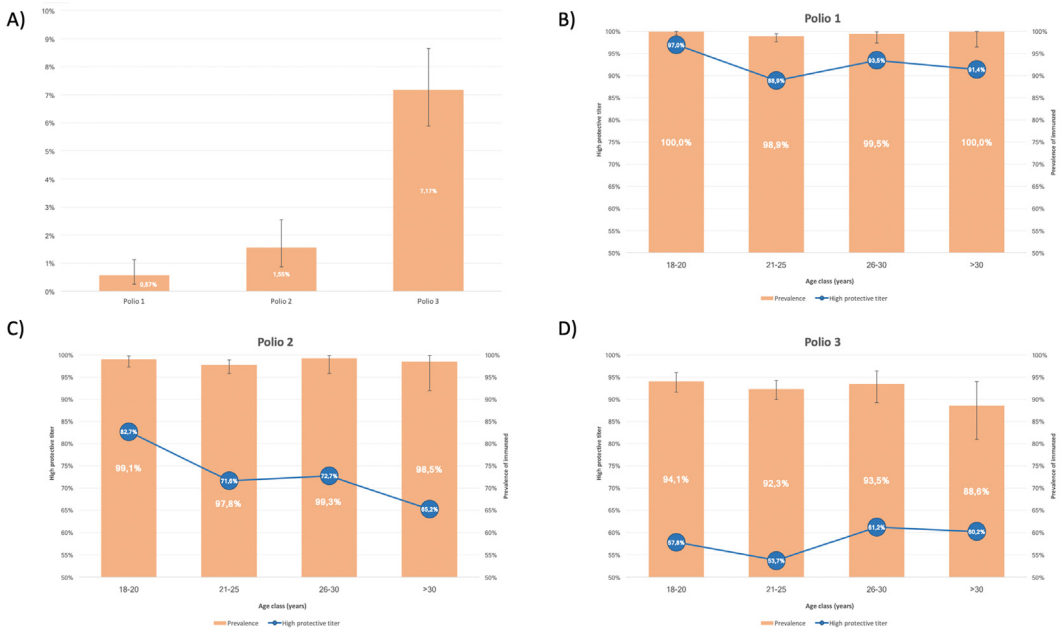

Fig. 1. (A) Prevalence (%) of study participants without neutralizing antibodies, per poliovirus type. (B) Prevalence (%) of study participants with neutralizing antibodies and of those with high protective titer against poliovirus 1. (C) Prevalence (%) of study participants with neutralizing antibodies and of those with a high protective titer against poliovirus 2. (D) Prevalence (%) of study participants with neutralizing antibodies and of those with a high protective titer against poliovirus 3.

Table 1
Proportion of study participants without polio neutralizing antibodies and the distribution of the titer level (low–high), including with respect to sex and poliovirus (PV) type.

Variable	PV1				PV2				PV3			
	Female (n = 915)	Male (n = 493)	Total (n = 1,408)	p-value	Female (n = 617)	Male (n = 350)	Total (n = 967)	p-value	Female (n = 915)	Male (n = 493)	Total (n = 1,408)	p-value
Susceptible; n (%; 95%CI)	7 (0.77; 0.31–1.57)	1 (0.20; 0.05–1.12)	8 (0.57; 0.25–1.12)	0.274	11 (1.78; 0.89–3.17)	4 (1.14; 0.31–2.90)	15 (1.55; 0.87–2.55)	0.591	65 (7.10; 5.53–8.97)	36 (7.30; 5.17–9.97)	101 (7.17; 5.88–8.65)	0.891
Protective titer level; n (%)				0.847				0,938				0.806
• low	68 (7.5)	38 (7.7)	106 (7.6)		152 (25.1)	86 (24.9)	238 (25.0)		366 (43.1)	200 (43.8)	566 (43.3)	
• high	840 (92.5)	454 (92.3)	1,294 (92.4)		454 (74.9)	260 (75.1)	714 /75.0)		484 (56.9)	257 (56.2)	741 (56.7)	

chi-squared and Fisher' exact tests were used to compare the proportions with respect to sex and age class.

To assess the determinants of seroprotection at the time of enrollment (seroconversion after the vaccine primary childhood series), multivariate logistic regression models were created for each type of poliovirus, in which seroprotection was the outcome and sex (male vs. female), age at enrollment (years), chronic disease (yes/no), and the time from the last vaccine dose to titer evaluation (years) were the determinants. Adjusted odds ratios (aORs) were calculated together with their 95%CIs.

Protective antibody survival (PAS), defined as the time elapsed from the last dose of routine OPV to the evaluation of the antibody titer (years), was determined and then evaluated using Kaplan-Meier curves. The log-rank test was used to evaluate the differences between sexes. The loss of seroprotection per 1,000 person-years and the 95%CIs were calculated. An incidence rate ratio (IRR), in which the value for females was the denominator and that for males the numerator, was also calculated together with the 95%CIs.

A multivariate Cox semiparametric regression was used to evaluate the determinants of PAS, with sex (male vs. female), age at enrollment (years), and chronic disease (yes/no) as risk predictors. The adjusted hazard ratio (aHR) and 95%CIs were determined as well. The Schoenfeld and scaled Schoenfeld residuals tests were used to evaluate the proportionality assumption of the multivariate Cox semiparametric regression model. The Gronnesby and Borgan test was used to evaluate the goodness-of-fit of the models.

For all the tests, a two-sided p-value < 0.05 was considered statistically significant.

3. Results

From April 2014 to October 2020, 6,105 medical students and residents were tested. The immunization status, downloaded from GIAVA, was available for 4,661/6,105 (76.3%). From this group, 1,408/4,661 (30.2%) had received four doses of OPV and were included in this study. Within the latter group, 915 (65.0%) were female. The average age at study enrollment was 23.1 ± 4.4 years (range = 18.0–51.0), without a difference between males (23.2 ± 4.2; range = 18–40) and females (23.1 ± 4.5; range = 18–51; p = 0.139). Chronic disease (heart disease, stroke, cancer, diabetes, chronic respiratory diseases, musculoskeletal and gastrointestinal disorders, vision and hearing defects, genetic diseases

and allergies) was reported in 584 of the 1,408 (41.5%) participants, again without a significant difference between males (n = 203/493; 41.2%) and females (n = 381/915; 41.6%; p = 0.886); no serious conditions were recorded.

All participants were tested for PV1 and PV 3 and 967 (68.7%) for PV2 (due to a lack of test reagent for a short time). None reported a history of polio.

3.1. PV1

The prevalence in the study population of the absence of neutralizing antibodies for PV1 was 0.57% (95%CI = 0.24–1.12; n = 8/1,408; Fig. 1A), without a sex-based difference (p > 0.05; Table 1). A high titer was detected in 92.4% (n = 1,294/1,408), without a difference between sexes (p > 0.05; Table 1). There were no differences in the seroprotection rate among the different age classes (p = 0.074), but the titer of neutralizing antibodies decreased significantly with increasing age (p < 0.0001; Fig. 1B). In the multivariate logistic regression there was no association between the seroprevalence of anti-PV1 antibodies and any of the analyzed determinants (p > 0.05; not shown).

The average PAS time was 20.8 ± 4.3 years (range = 11–46). The incidence of seronegativity per 1,000 person-years was 0.27 (95% CI = 0.13–0.55) and was lower in males (0.10; 95%CI = 0.01–0.69) than in females (0.37; 95%CI = 0.17–0.78), with an IRR of 0.26 (95%CI = 0.01–2.04; p = 0.195). There was no significant sex-based difference in the PAS (logrank p = 0.145; Fig. 2A). In the multivariate Cox semiparametric regression, the only predictor of PAS was age but not sex or chronic disease status (Table 2).

3.2. PV2

PV2 neutralizing antibodies were not detectable in 1.56% (95% CI = 0.87–2.55; n = 15/967; Fig. 1) of the study population, without a difference between males and females (p > 0.05; Table 1). A high titer was determined in 75.0% (n = 714/952), again without a sex-based difference (p > 0.05; Table 1). Immunoprotection rates did not differ significantly among the different age classes of the study population (p = 0.436), but the proportion with a high titer of neutralizing antibodies decreased with increasing age (p = 0.001; Fig. 1C). In the multivariate logistic regression there was no association between the seroprevalence of anti-PV2 antibodies and any of the analyzed determinants (p > 0.05; not shown).

The average PAS time was 20.6 ± 4.2 years (range = 12–46). The incidence of seronegativity per 1,000 person-years was 0.51 (95% CI = 0.31–0.85) and was lower in males (0.39; 95%CI = 0.15–1.03) than in females (0.58; 95%CI = 0.32–1.05), with an IRR of 0.67 (95%CI = 0.15–2.24; p = 0.508). The PAS did not differ as a function of sex (logrank p = 0.397; Fig. 2B). In the multivariate Cox semiparametric regression, the only predictor of PAS was age but not sex or chronic disease status (Table 2).

3.3. PV3

Within the study population, 7.17% (95%CI = 5.88–8.65; n = 101/1,408; Fig. 1) had no evidence of PV3 neutralizing antibodies, without a sex-based difference (p > 0.05; Table 1). A high titer was detected in 56.7% (n = 741/1,307), without a difference between males and females (p > 0.05; Table 1). There was also no difference in the immunoprotection rate with respect to age class (p = 0.220) or among the participants with a high titer of neutralizing antibodies (p = 0.217; Fig. 1D). In the multivariate logistic regression analysis, there was no association between the seroprevalence of anti-PV3 antibodies and any of the analyzed determinants (p > 0.05; not shown).

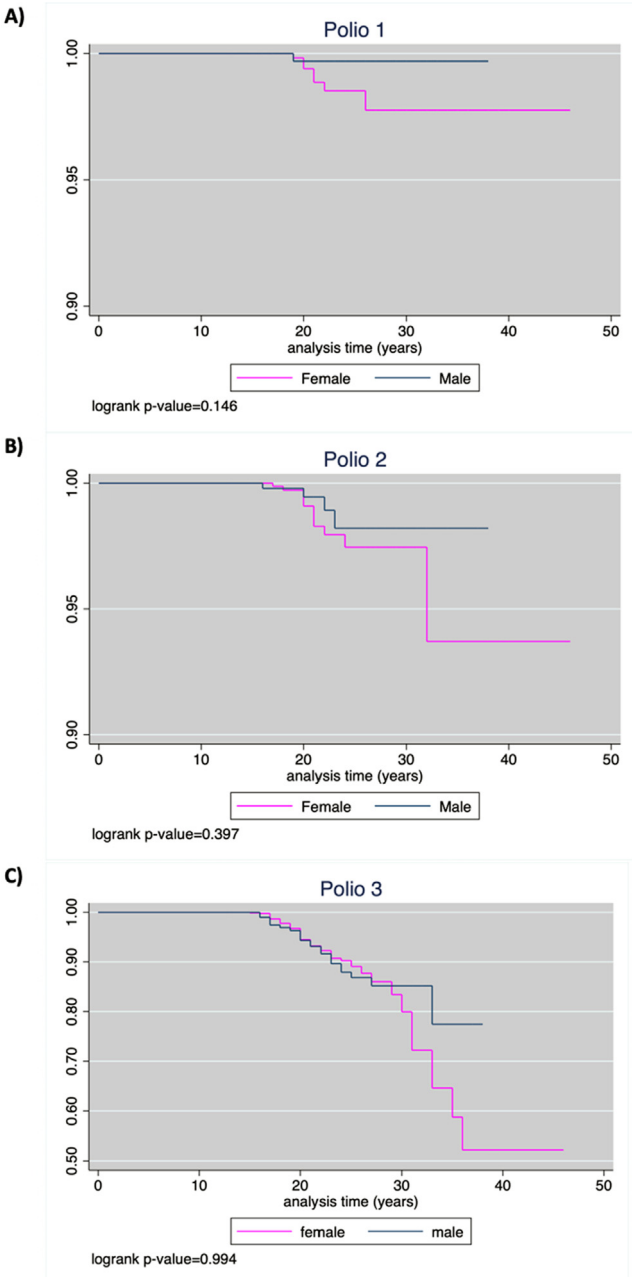

Fig. 2. Klapan-Meier protective antibody survival (PAS) estimates, per sex for poliovirus (PV)1 (A), PV2 (B), and PV3 (C).

The average PAS time was 20.8 ± 4.3 years (range = 11–46). The incidence of seronegativity per 1,000 person-years was 3.42 (95% CI = 2.81–4.16) and was similar in males (3.48; 95%CI = 2.51–4.83) and females (3.38; 95%CI = 2.65–4.32), with an IRR of 1.03 (95%CI = 0.66–1.57; p = 0.994). The PAS also did not differ with in males and females (logrank p = 0.994; Fig. 2C). In the multivariate Cox semiparametric regression, the only predictor of PAS was age but not sex or chronic disease status (Table 2).

No subject negative to PV1 resulted to be negative to PV2 or PV3.

Table 2
Multivariate Cox semiparametric regression analysis of the risk predictors of protective antibody survival per poliovirus type.

Risk predictor	PV1		PV2		PV3	
	aHR (95%CI)	p-value	aHR (95%CI)	p-value	aHR (95%CI)	p-value
Sex (male vs. female)	0.16 (0.02–1.60)	0.119	0.47 (0.14–1.59)	0.226	0.87 (0.57–1.32)	0.506
Age (years)	0.42 (0.24–0.73)	0.002	0.41 (0.29–0.59)	0.000	0.40 (0.35–0.47)	0.000
Chronic disease (yes/no)	0.52 (0.10–2.60)	0.422	1.36 (0.48–3.88)	0.566	1.19 (0.79–1.79)	0.400
Goodness-of-fit	**X^2 = 3.4; p = 0.063**		**X^2 = 3.4; p = 0.065**		**X^2 = 0.9; p = 0.356**	

4. Conclusions

Our study showed that, among a population fully vaccinated with the OPV, neutralizing antibodies against all three types of poliovirus were present in > 90%, with rates of > 99% for PV1, >98% for PV2, and almost 93% for PV3. A 2008 Italian study [18] of Apulian children (vaccination status unknown) and adolescents determined seropositivity rates of > 99% for all three viruses. The older age of our study's participants may explain the slightly lower antibody titers, especially for PV3. A 2018 Italian study [21] of vaccinated (but immunized according to different vaccination schedules) adolescent and adults (age range: 12–50 years) reported a seroprotection rate of 92.9%, 96.2%, and 83.4%, for PV1, PV2, and PV3, respectively, similar to our results (although the rate for PV1 was significantly higher in our study). In another Italian serosurvey [22] from 2009, immunity against PV1, PV2, and PV3 was evaluated in an immunocompetent population (n = 328; mean age 38 years, range: 0–88 years) with an unknown vaccination status. The protective rate for PV1, PV2, and PV3 was 75.3%, 69.2%, and 46%, respectively, suggesting the need for a fifth IPV dose in the Italian routine schedule.

None of our analyses indicated that sex affected the seropositivity rate or the immune response to OPV. However. sex-based differences in the response to vaccines has been the focus of several studies [267–270], all of which found that females are more responsive to vaccines and or infection than males.

Although in our study serosusceptibility did not significantly decrease with increasing age, a decrease in the levels of neutralizing antibodies with increasing age was apparent, especially for PV1 and PV2. This decline is a proxy for the real risk factor, which is the time elapsed since the last dose of vaccine, as also shown by the semiparametric Cox regression models. Over time, OPV seems to trigger an immune response that leads to higher levels of neutralizing antibodies for PV1 (89–97%), lower levels for PV2 (65–83%), and even lower levels for PSV3 (54–61%). Like other vaccines [19,23–26], a role for age (or time elapsed since the last dose) in the response to polio vaccines has been demonstrated in many investigations [18,21,27,28].

The PAS analysis showed that protective antibodies against all three viruses persist for at least 18 years after the administration of the last dose of OPV, evidenced by the fact that > 95% of our study population had PV1 and PV2 antibodies and ~ 50% had PV3 antibodies > 30 years after the last dose of OPV. Although the long duration of OPV immunization is well established [17], to our knowledge ours is the first study to provide a quantitative assessment in a large study population vaccinated with four doses during childhood. Our findings should be considered in light of the absence of natural boosters in Italy, where in the last 30 years there has been no case of polio. In addition, in the Apulia region, analyses of stool samples from arriving refugees have likewise found no evidence of poliovirus circulation [29].

In summary, the time between the last vaccination against polio and the antibody titer evaluation is a determinant of the levels of persisting neutralizing antibodies. Although the antibody titer decreases over the years, immunity against PV1 and PV2 can be considered life-long.

The strengths of our study were the large sample size, its being one of the few studies of the long-term immunogenicity of OPV, and the comparisons based on sex and age class. Nonetheless, a major limitation was the age distribution of the study participants, as most were < 25 years of age, which could have distorted the results since young adults have better immunity memory. In future years, further studies should clarify if the low antibody level is related to the loss of protection, or if the decline of the titer makes antibodies undetectable without putting subjects at risk.

Geographically, Apulia is a border region that in recent years has taken in a large number of refugees, such that there is a risk of poliovirus importation from endemic areas, even if a 2015 study [30] reported good level of immunization against polio among refugees. The situation in the rest of Italy is similar. However, as long as there are areas of the world where polio is still present, its reappearance elsewhere in the world is possible. Consequently, there is active surveillance in Italy for cases of acute flaccid paralysis, one of the most serious complications of poliomyelitis. All cases of acute flaccid paralysis, of any etiology, in patients under the age of 15 and all cases of suspected poliomyelitis regardless of patient age must be reported [31]. Furthermore, a high level of polio vaccine coverage remains an objective of the most recent Italian immunization plan [32].

In conclusion, the primary childhood series of OPV guarantees long-lasting protection. PV2 and PV3 have been eradicated, and protection against PV1 remains close to 100% even after many years. Therefore, a booster dose of vaccine (IPV) after the primary childhood series cannot be recommended, as suggested by some studies [22,28]. However, until the complete eradication of PV1, public health institutions must be alert to its possible appearance (in fact, stocks of OPV vaccine are ready in case of reintroduction of the wild virus in European territory [33]). Thus far, the efficacy of the vaccination campaign implemented beginning in the 1960s has freed the world from a disease that plagued humanity for centuries.

Future studies should focus on the long-term immunogenicity of mixed vaccination schedules and, above all, on the current four-dose schedule for IPV (plus an additional dose in adolescence), to evaluate any critical issues that may lead to a risk situation in the event of reintroduction of the wild virus.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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